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Biological effects of drug-eluting stents in the coronary circulation

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Abstract: Drug-eluting stents (DES) are designed to release pharmacological agents into the vessel wall in order to inhibit the response to injury causing restenosis, i.e., vascular smooth muscle cell migration and proliferation. Once deployed, however, these substances exert many biological effects in the coronary circulation; their action is indeed not confined to inhibition of vascular smooth muscle cells, but extends to other cell types such as endothelial cells. Both rapamycin and paclitaxel decrease endothelial cell migration and proliferation; moreover, they induce tissue factor expression through specific interaction with signal transduction mediators. As both effects would lead to an increased thrombogenic potential of DES, they appear particularly important in light of a possibly increased risk for stent thrombosis with DES as compared to bare-metal stents. This aspect is further highlighted by the observation that DES also decrease proliferation, differentiation, and homing of endothelial progenitor cells, which are believed to contribute to reendothelialization after stent implantation. Furthermore, both rapamycin and paclitaxel have been demonstrated to induce endothelial dysfunction in the coronary vasculature distal to the stent. Finally, the polymer used for DES may be associated with hypersensitivity reactions, which may, at least in some cases, favor stent thrombosis. This review will discuss the biological effects of DES in the coronary vasculature

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Key Words:

Drug-eluting stent · Restenosis · Thrombosis · Tissue factor · Endothelial regeneration

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Biological Effects of Drug-Eluting Stents in the Coronary Circulation

Jan Steffel, Felix C. Tanner¹

Abstract

Drug-eluting stents (DES) are designed to release pharmacological agents into the vessel wall in order to inhibit the response to injury causing restenosis, i.e., vascular smooth muscle cell migration and proliferation. Once deployed, however, these substances exert many biological effects in the coronary circulation; their action is indeed not confined to inhibition of vascular smooth muscle cells, but extends to other cell types such as endothelial cells. Both rapamycin and paclitaxel decrease endothelial cell migration and proliferation; moreover, they induce tissue factor expression through specific interaction with signal transduction mediators. As both effects would lead to an increased thrombogenic potential of DES, they ap-

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Biologische Effekte von Drug-eluting-Stents im koronaren Gefäßbett**Schlüsselwörter:**

Drug-eluting-Stents · Restenose · Thrombose · Tissue-Faktor · Endotheliale Regeneration

Zusammenfassung

Drug-eluting-Stents (DES) setzen nach ihrer Implantation pharmakologische Substanzen in die Gefäßwand frei, welche die zur Restenose führenden Prozesse – Migration und Proliferation glatter Gefäßmuskulzellen – inhibieren. Die Wirkung dieser Pharmaka ist jedoch vielfältig und zudem nicht auf glatte Gefäßmuskulzellen beschränkt; so inhibieren sowohl Rapamycin als auch Paclitaxel die Migration und Proliferation von Endothelzellen und induzieren außerdem die Expression von Tissue-Faktor durch spezifische Interaktion mit Mediatoren der Signaltransduktion. Da diese beiden Effekte zu einer erhöhten Stentthrombogenität führen können, erscheinen sie von großer Wichtigkeit, insbesondere im Rahmen der aktuellen Diskussion um eine möglicherweise er-

höhte Rate von Stentthrombosen bei DES im Vergleich zu unbeschichteten Bare-Metal-Stents. Hinzu kommt, dass sowohl Rapamycin als auch Paclitaxel die Proliferation, Migration und Differenzierung von endothelialen Progenitorzellen, welche ebenfalls zur Reendothelialisierung nach Stentimplantation beizutragen scheinen, hemmen. Darüber hinaus konnte gezeigt werden, dass beide Substanzen in Gefäßsegmenten distal der gestenteten Läsion eine endotheliale Dysfunktion induzieren. Schließlich kann das Polymer, welches zur Beschichtung verwendet wird, zu Hypersensitivitätsreaktionen in der Gefäßwand führen, was zumindest in manchen Fällen die Entstehung einer Stentthrombose zu begünstigen scheint. Dieser Übersichtsartikel beleuchtet die biologischen Effekte von DES im koronaren Gefäßbett.

Introduction

With the introduction of balloon-expandable bare-metal stents (BMS), coronary remodeling and in turn restenosis rates were reduced as compared to angioplasty alone [16, 44]. As the risk of restenosis remained > 10%, however, drug-eluting stents (DES) were designed which release pharmacological agents after deployment. On first-generation DES, rapamycin (sirolimus, used on Cypher® stents) or paclitaxel (used on Taxus® stents) were applied in order to re-

duce restenosis through inhibition of vascular smooth muscle cell (VSMC) migration and proliferation. As a result, the rates of restenosis and target vessel revascularization could be reduced to < 10% after DES implantation [37, 38].

Already in the first series of patients receiving BMS, however, stent thrombosis was recognized as a severe complication owing to its high mortality. Indeed, despite reduced restenosis rates, the incidence of in-stent thrombosis has not decreased with DES as

compared to BMS [3, 4, 8, 35, 36]. Several hundred cases of stent thrombosis have been reported for rapamycin-coated stents [32], and a number of reports imply that thrombosis rates of DES may even be higher in “real-world” patients than in clinical trials [27, 40].

This review article focuses on the biological effects of DES in the coronary circulation.

Biological Effects of Drug-Eluting Stents in the Coronary Circulation

DES Impair VSMC Migration and Proliferation

Agents released from DES inhibit migration and proliferation of VSMCs, which are the crucial events in neointima formation and the development of restenosis (Figure 1) [33, 42]. In vitro, VSMC proliferation is blocked by rapamycin via interference with several cell-cycle regulators involved in G_1 -S phase transition including p27^{Kip1} [31], p21^{cip1} [30], cyclin D isoforms, and, finally, the retinoblastoma protein (pRb) [33]. Although not fully resolved in detail, the antimigratory effect of rapamycin appears to occur via interaction with p27^{Kip1} as well [50]. In vivo, rapamycin inhibits intimal thickening after balloon angioplasty in porcine and rodent animal models through interaction with cell-cycle regulators [17, 43]. The role of p27^{Kip1} in this process, however, remains unclear, as some studies imply an effect of rapamycin on this protein [17], while others do not [6, 43].

Everolimus, an orally active compound of the sirolimus family, is used on second-generation DES (Xience®); when applied orally in a rabbit iliac artery model, it significantly reduces neointimal growth [14]. Similarly, zotarolimus, a synthetic rapamycin analog developed specifically for stent application (Endeavor®), inhibits VSMC migration and proliferation and reduces neointima formation in a porcine coronary artery model [18].

Paclitaxel, a lipophilic diterpenoid that binds to the β -subunit of the tubulin heterodimer, stabilizes microtubules, induces cell-cycle arrest in G_2 -M phase, and, eventually, inhibits VSMC migration and proliferation [2, 11, 45]. Consistently, an inhibition of neointima formation is observed in animal models after local delivery of paclitaxel [25].

DES Inhibit Endothelial Cell Migration and Proliferation

Coronary angioplasty leads to disruption of the endothelial layer leaving a highly prothrombotic surface exposed to the blood stream. Restoration of an intact endothelium therefore represents a crucial process in reestablishing an antithrombotic vessel surface. Traditionally, it is believed that reendothelialization oc-

curs after vascular injury and stent deployment because endothelial cells proliferate and migrate from intact neighboring coronary segments, eventually leading to endothelial healing of the injured segment.

Given the ubiquitous expression of cell-cycle regulatory proteins, it is conceivable that agents released from DES do not only affect proliferation and migration of VSMCs, but also of endothelial cells (Figure 1). Indeed, rapamycin potently inhibits endothelial cell proliferation [48] and migration [34] in vitro. Similarly, paclitaxel reduces endothelial cell proliferation and migration through interaction with cell-cycle regulators and, at least in part, through induction of apoptosis [41].

In animal models, the time course of reendothelialization after stent implantation varies in different species. In pigs, the extent of reendothelializa-

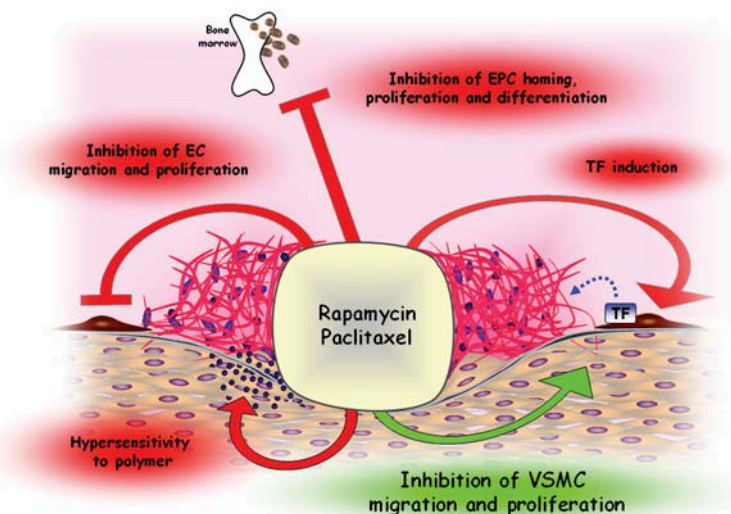
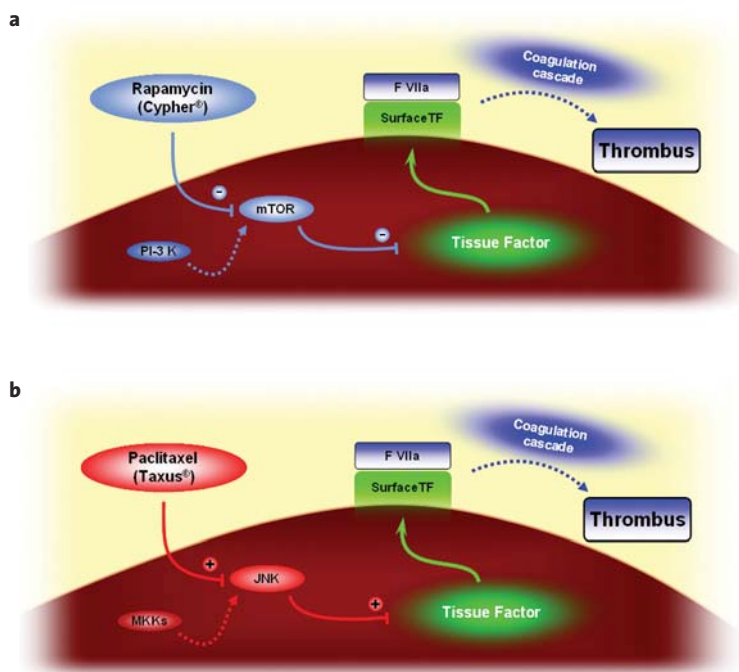


Figure 1. Biological effects of drug-eluting stents (DES) in the coronary circulation.

Simplified overview of the effect of a DES strut on the vessel wall after deployment. Sirolimus/paclitaxel inhibit vascular smooth muscle cell (VSMC) migration and proliferation, thereby reducing restenosis. On the other hand, these drugs inhibit migration and proliferation of neighboring endothelial cells (EC), induce tissue factor (TF) expression, and inhibit homing, proliferation, and differentiation of endothelial progenitor cells (EPC), potentially leading to an increase in stent thrombogenicity. Moreover, the polymer or the stent strut itself may elicit a hypersensitivity reaction in the vessel wall.

Abbildung 1. Biologische Effekte von Drug-eluting-Stents (DES) in der Koronarzirkulation.

Vereinfachter Überblick über den Effekt von DES auf die Gefäßwand nach Implantation. Sirolimus/Paclitaxel inhibieren die Migration und Proliferation glatter Gefäßmuskulzellen (VSMC) und reduzieren somit die Restenose. Auf der anderen Seite inhibieren diese Medikamente ebenfalls die Migration und Proliferation von angrenzenden Endothelzellen (EC); des Weiteren induzieren sie die Expression von Tissue-Faktor (TF) und inhibieren das „homing“, die Proliferation sowie die Differenzierung endothelialer Vorläuferzellen (EPC), was potentiell die Stent-thrombogenität erhöht. Darüber hinaus kann das Polymer oder das Stentgerüst selbst eine Hypersensitivitätsreaktion in der Gefäßwand auslösen.



Figures 2a and 2b. Rapamycin and paclitaxel increase tissue factor (TF) expression.

a) Rapamycin binds to the mammalian target of rapamycin (mTOR); mTOR is a downstream mediator of the phosphatidylinositol-3 kinase (PI-3K) pathway, which plays an inhibitory role in TF expression. Hence, by inhibiting mTOR, rapamycin leads to an increase in TF protein expression and surface activity. b) Paclitaxel increases c-jun NH₂-terminal kinase (JNK) activation leading to an enhanced TF protein expression as well as surface activity. Thus, paclitaxel directly activates a positive regulatory pathway of TF expression, while rapamycin upregulates TF by disinhibiting an inhibitory pathway. MKKs: MAP kinase kinases.

Abbildungen 2a und 2b. Rapamycin und Paclitaxel erhöhen die Expression von Tissue-Faktor (TF).

a) Rapamycin bindet an mTOR („mammalian target of rapamycin“); mTOR ist ein Downstream-Mediator des Phosphatidylinositol-3-Kinase-(PI-3K-)Signaltransduktionswegs, welcher eine inhibitorische Rolle in der TF-Induktion spielt. Über die Hemmung von mTOR führt Rapamycin somit zu einer Zunahme der TF-Proteinexpression und Oberflächenaktivität. b) Paclitaxel verstärkt die Aktivierung von JNK („c-jun NH₂-terminal kinase“), welche zu einer Erhöhung der Proteinexpression und Oberflächenaktivität von TF führt. Paclitaxel aktiviert somit direkt einen positiven Signaltransduktionsweg, während Rapamycin TF durch die Disinhibition eines hemmenden Signaltransduktionswegs induziert. MKKs: „MAP kinase kinases“.

tion is similar between BMS and DES after 28 days [51]; however, delayed endothelial healing occurs in a rabbit iliac overlapping stent model after implantation of sirolimus- or paclitaxel-eluting stents as compared to BMS [15]. In humans, near complete reendothelialization seems to occur by 3–4 months after BMS implantation [13]. In an autopsy study comparing coronary artery segments after implantation of DES with BMS, delayed arterial healing and poorer reendothelialization are documented after DES implantation [29]. Hence, there is evidence that the currently used DES may significantly im-

pair reendothelialization after coronary artery stent deployment.

DES Inhibit Homing, Proliferation, and Differentiation of Endothelial Progenitor Cells

Endothelial progenitor cells (EPCs) seem to be involved in reendothelialization after angioplasty [19, 53]; hence, it is important to understand the effect of DES on EPC biology. Rapamycin indeed inhibits proliferation, migration, and differentiation of human EPCs in vitro [7, 10]; these effects occur via interaction with the mammalian target of rapamycin (mTOR) and, at least in part, by induction of apoptosis. It therefore comes as no surprise that, in contrast to BMS, implantation of a sirolimus-eluting stent leads to a decrease in circulating CD34-positive cells [28]. Hence, drugs loaded on DES may affect homing, proliferation, and differentiation of EPCs thereby delaying proper endothelial regeneration (Figure 1).

DES Induce Endothelial Tissue Factor Expression

On a molecular level, rapamycin binds to the FK-binding protein 12 (FKBP-12) and thereby inhibits phosphorylation of mTOR. mTOR is a downstream target of the phosphatidylinositol-3 kinase (PI-3K) pathway, which in turn regulates translation by phosphorylation of p70S6 ribosomal protein kinase (p70S6K) [24]. The inhibitory role of the PI-3K pathway on expression of endothelial tissue factor (TF) is well established, as its inhibition enhances TF expression in response to thrombin or tumor necrosis factor-(TNF)- α [9, 12]. Consistently, inhibition of mTOR by rapamycin increases thrombin- as well as TNF- α -induced endothelial TF expression and activity (Figure 2a) [48]. Importantly, this effect occurs at rapamycin concentrations observed in vivo after stent implantation [48, 54]. The effect of rapamycin on TF expression in VSMCs is controversial: while we did not observe an effect after 5 h of incubation [48], prolonged exposition (e.g., 24 h) of rapamycin has been demonstrated to slightly elevate VSMC TF expression [57].

Paclitaxel does not only stabilize microtubules, but also activates signal transduction molecules such as JNK [46, 55], an important mediator of endothelial TF induction [20, 47, 49]. Consequently, paclitaxel increases endothelial TF expression and activity (Figure 2b) [46]; again, the concentrations exerting this effect are comparable with local tissue concentrations encountered after stent deployment [15].

Sirolimus-eluting stents are designed in a way that about 80% of the loaded rapamycin has eluted by 30 days [37, 38]. By contrast, paclitaxel-eluting

stents have a biphasic drug release profile in vitro with an initial burst during the first 48 h after implantation followed by a sustained low-level release for at least 2 weeks [23]. Owing to their lipophilic properties, both rapamycin and paclitaxel easily penetrate cell walls leading to retention of the drugs in the arterial tissue [15, 21, 51]. Thus, TF induction in response to both rapamycin and paclitaxel may promote a prothrombotic environment after deployment of DES (Figure 1). In how far these findings truly translate into clinical practice, however, requires further studies, in particular with respect to the extent and the spatiotemporal pattern of TF induction in the arterial wall after DES deployment.

DES Induce Endothelial Dysfunction in the Distal Coronary Circulation

There is evidence that both rapamycin and paclitaxel may not only affect the vasculature at the site of stent implantation, but also in coronary artery segments distal to the stent. Indeed, long-term endothelial dysfunction of coronary artery segments distal to sirolimus-eluting stent, but not BMS, occurs as indicated by significant vasoconstriction in response to acetylcholine infusion [26]. Similarly, implantation of paclitaxel-eluting stents is associated with exercise-induced vasoconstriction in the segments proximal and distal to the stent, while exercise-induced vasodilation is observed after BMS placement [52]. Taken together, these findings indicate that drugs released from DES may affect the distal coronary vasculature facilitating coronary vasoconstriction and potentially contributing to no-reflow phenomena after stent deployment.

Effect of the Stent Material in the Coronary Circulation

Certain stent materials appear to promote the development of stent thrombosis. For example, implantation of an open-cell versus a closed-cell stent resulted in greater platelet activation 30 days following implantation [22]. The stent strut thickness as well as the polymer type also appear to play an important role. Chronic eosinophilic infiltration of the arterial wall suggestive of a hypersensitivity reaction has also been observed, probably in response to the nonerodable polymers of DES [29, 39]; mostly, these reactions occur > 4 months after DES implantation (Figure 1) [29, 32]. While the polymer may have an effect on the coronary vasculature after DES placement, the causal relationship between polymer-induced inflammation and the incidence of late stent thrombosis has only been suggested in a minority of patients.

Design of Future Drug-Eluting Stents

The ideal DES inhibits restenosis without interfering with (or even expediting) reendothelialization, all on the basis of a biologically inert polymer. While several novel approaches aim at improving currently available DES, no ideal combination of materials and drugs has yet been developed. One potential approach uses a simple chemical coating, such as titanium-nitride oxide, which diminishes platelet adhesion and fibrinogen binding as compared to BMS; whether this strategy will be effective against restenosis and stent thrombosis, however, remains to be studied in large clinical trials [56]. Coating of stents with agents facilitating reendothelialization after stent implantation may represent another feasible approach to reduce the thrombogenicity of DES. In a porcine coronary artery stenting model, stents loaded with an integrin-binding cyclic Arg-Gly-Asp peptide indeed accelerate endothelialization by attracting EPCs [5]. In humans, endothelial coverage is accelerated after implantation of stents coated with antibodies against CD34 designed to capture EPCs [1]. However, further studies are needed to assess the long-term efficacy and safety of these biologically active stents. A combination of substances accelerating endothelial healing (such as vascular endothelial growth factor and others) with established agents reducing restenosis represents another promising approach.

Dimethyl sulfoxide (DMSO) may also represent an interesting therapeutic principle for DES coating. We recently demonstrated that DMSO prevents VSMC proliferation and migration; at the same time, DMSO reduces upregulation of TF in endothelial cells, vascular smooth muscle, and monocytes, and prevents thrombotic occlusion in vivo in a mouse carotid artery injury model [9]. Thus, DMSO may have the potential to inhibit both neointima formation and stent thrombosis, if loaded on a DES. Whether these promising first results hold true in a larger animal model and in humans, however, remains to be determined.

Summary and Conclusion

Once deployed, DES exert different biological effects in the coronary circulation. Importantly, their action is not confined to inhibition of migration and proliferation of VSMCs, i.e., important factors in neointima formation and the development of restenosis, but extends to other cell types such as endothelial cells. Indeed, both rapamycin and paclitaxel decrease endothelial cell migration and proliferation; in addition, the drugs induce endothelial TF expression through specific interaction with signal transduction mediators. Furthermore, DES decrease homing, proliferation, and differentiation of EPCs, equally believed to be involved in endothelial regeneration af-

ter stent implantation. Both rapamycin and paclitaxel have also been demonstrated to induce endothelial dysfunction in the distal coronary vasculature. Finally, the polymer used for DES may be associated with hypersensitivity reactions which may, at least in some cases, promote stent thrombosis.

These undesired local side effects of DES in the vessel wall clearly demonstrate the need for improvement of currently applied first-generation DES; indeed, these side effects appear particularly important in the light of the ongoing debate of a possibly increased risk of stent thrombosis with DES as compared to BMS. Hence, improvements of currently available systems as well as the introduction of novel treatment strategies are needed in order to advance the development toward the goal of preventing restenosis without the cost of delayed endothelial healing and increased stent thrombogenicity.

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References

- Aoki J, Serruys PW, van Beusekom H, et al. Endothelial progenitor cell capture by stents coated with antibody against CD34: the HEALING-FIM (Healthy Endothelial Accelerated Lining Inhibits Neointimal Growth-First In Man) Registry. *J Am Coll Cardiol* 2005;45:1574–9.
- Axel DI, Kunert W, Goggelmann C, et al. Paclitaxel inhibits arterial smooth muscle cell proliferation and migration in vitro and in vivo using local drug delivery. *Circulation* 1997;96:636–45.
- Bavry AA, Kumbhani DJ, Helton TJ, et al. Risk of thrombosis with the use of sirolimus-eluting stents for percutaneous coronary intervention (from registry and clinical trial data). *Am J Cardiol* 2005;95:1469–72.
- Bavry AA, Kumbhani DJ, Helton TJ, et al. What is the risk of stent thrombosis associated with the use of paclitaxel-eluting stents for percutaneous coronary intervention? A meta-analysis. *J Am Coll Cardiol* 2005;45:941–6.
- Blindt R, Vogt F, Astafieva I, et al. A novel drug-eluting stent coated with an integrin-binding cyclic Arg-Gly-Asp peptide inhibits neointimal hyperplasia by recruiting endothelial progenitor cells. *J Am Coll Cardiol* 2006;47:1786–95.
- Braun-Dullaes RC, Mann MJ, Seay U, et al. Cell cycle protein expression in vascular smooth muscle cells in vitro and in vivo is regulated through phosphatidylinositol 3-kinase and mammalian target of rapamycin. *Arterioscler Thromb Vasc Biol* 2001;21:1152–8.
- Butzal M, Loges S, Schweizer M, et al. Rapamycin inhibits proliferation and differentiation of human endothelial progenitor cells in vitro. *Exp Cell Res* 2004;300:65–71.
- Camenzind E, Steg PG, Wijns W. Stent thrombosis late after implantation of first-generation drug-eluting stents: a cause for concern. *Circulation* 2007;115:1440–55.
- Camici GG, Steffel J, Akhmedov A, et al. Dimethyl sulfoxide inhibits tissue factor expression, thrombus formation, and vascular smooth muscle cell activation: a potential treatment strategy for drug-eluting stents. *Circulation* 2006;114:1512–21.
- Chen TG, Chen JZ, Wang XX. Effects of rapamycin on number activity and eNOS of endothelial progenitor cells from peripheral blood. *Cell Prolif* 2006;39:117–25.
- Crown J, O'Leary M. The taxanes: an update. *Lancet* 2000;355:1176–8.
- Eto M, Kozai T, Cosentino F, et al. Statin prevents tissue factor expression in human endothelial cells: role of Rho/Rho-kinase and Akt pathways. *Circulation* 2002;105:1756–9.
- Farb A, Burke AP, Kolodgie FD, et al. Pathological mechanisms of fatal late coronary stent thrombosis in humans. *Circulation* 2003;108:1701–6.
- Farb A, John M, Acampado E, et al. Oral everolimus inhibits in-stent neointimal growth. *Circulation* 2002;106:2379–84.
- Finn AV, Kolodgie FD, Harnek J, et al. Differential response of delayed healing and persistent inflammation at sites of overlapping sirolimus- or paclitaxel-eluting stents. *Circulation* 2005;112:270–8.
- Fischman DL, Leon MB, Baim DS, et al. A randomized comparison of coronary-stent placement and balloon angioplasty in the treatment of coronary artery disease. Stent Restenosis Study Investigators. *N Engl J Med* 1994;331:496–501.
- Gallo R, Padurean A, Jayaraman T, et al. Inhibition of intimal thickening after balloon angioplasty in porcine coronary arteries by targeting regulators of the cell cycle. *Circulation* 1999;99:2164–70.
- Garcia-Touchard A, Burke SE, Toner JL, et al. Zotarolimus-eluting stents reduce experimental coronary artery neointimal hyperplasia after 4 weeks. *Eur Heart J* 2006;27:988–93.
- Griese DP, Ehsan A, Melo LG, et al. Isolation and transplantation of autologous circulating endothelial cells into denuded vessels and prosthetic grafts: implications for cell-based vascular therapy. *Circulation* 2003;108:2710–5.
- Guha M, Mackman N. The phosphatidylinositol 3-kinase-Akt pathway limits lipopolysaccharide activation of signaling pathways and expression of inflammatory mediators in human monocytic cells. *J Biol Chem* 2002;277:32124–32.
- Gummert JF, Ikonen T, Morris RE. Newer immunosuppressive drugs: a review. *J Am Soc Nephrol* 1999;10:1366–80.
- Gurbel PA, Callahan KP, Malinin AI, et al. Could stent design affect platelet activation? Results of the Platelet Activation in STenting (PAST) study. *J Invasive Cardiol* 2002;14:584–9.
- Halkin A, Stone GW. Polymer-based paclitaxel-eluting stents in percutaneous coronary intervention: a review of the TAXUS trials. *J Intervent Cardiol* 2004;17:271–82.
- Hay N, Sonenberg N. Upstream and downstream of mTOR. *Genes Dev* 2004;18:1926–45.
- Heldman AW, Cheng L, Jenkins GM, et al. Paclitaxel stent coating inhibits neointimal hyperplasia at 4 weeks in a porcine model of coronary restenosis. *Circulation* 2001;103:2289–95.
- Hofma SH, van der Giessen WJ, van Dalen BM, et al. Indication of long-term endothelial dysfunction after sirolimus-eluting stent implantation. *Eur Heart J* 2006;27:166–70.
- Iakovou I, Schmidt T, Bonizzoni E, et al. Incidence, predictors, and outcome of thrombosis after successful implantation of drug-eluting stents. *JAMA* 2005;293:2126–30.
- Inoue T, Sata M, Hikichi Y, et al. Mobilization of CD34-positive bone marrow-derived cells after coronary stent implantation: impact on restenosis. *Circulation* 2007;115:553–61.
- Joner M, Finn AV, Farb A, et al. Pathology of drug-eluting stents in humans: delayed healing and late thrombotic risk. *J Am Coll Cardiol* 2006;48:193–202.

30. Li JM, Brooks G. Cell cycle regulatory molecules (cyclins, cyclin-dependent kinases and cyclin-dependent kinase inhibitors) and the cardiovascular system; potential targets for therapy? *Eur Heart J* 1999;20:406–20.
31. Luo Y, Marx SO, Kiyokawa H, et al. Rapamycin resistance tied to defective regulation of p27Kip1. *Mol Cell Biol* 1996; 16:6744–51.
32. Luscher TF, Steffel J, Eberli FR, et al. Drug-eluting stent and coronary thrombosis: biological mechanisms and clinical implications. *Circulation* 2007;115:1051–8.
33. Marx SO, Jayaraman T, Go LO, et al. Rapamycin-FKBP inhibits cell cycle regulators of proliferation in vascular smooth muscle cells. *Circ Res* 1995;76:412–7.
34. Matter CM, Rozenberg I, Jaschko A, et al. Effects of tacrolimus or sirolimus on proliferation of vascular smooth muscle and endothelial cells. *J Cardiovasc Pharmacol* 2006;48: 286–92.
35. McFadden EP, Stabile E, Regar E, et al. Late thrombosis in drug-eluting coronary stents after discontinuation of antiplatelet therapy. *Lancet* 2004;364:1519–21.
36. Moreno R, Fernandez C, Hernandez R, et al. Drug-eluting stent thrombosis: results from a pooled analysis including 10 randomized studies. *J Am Coll Cardiol* 2005;45:954–9.
37. Morice MC, Serruys PW, Sousa JE, et al. A randomized comparison of a sirolimus-eluting stent with a standard stent for coronary revascularization. *N Engl J Med* 2002;346:1773–80.
38. Moses JW, Leon MB, Popma JJ, et al. Sirolimus-eluting stents versus standard stents in patients with stenosis in a native coronary artery. *N Engl J Med* 2003;349:1315–23.
39. Nebeker JR, Virmani R, Bennett CL, et al. Hypersensitivity cases associated with drug-eluting coronary stents: a review of available cases from the Research on Adverse Drug Events and Reports (RADAR) project. *J Am Coll Cardiol* 2006;47:175–81.
40. Ong AT, McFadden EP, Regar E, et al. Late angiographic stent thrombosis (LAST) events with drug-eluting stents. *J Am Coll Cardiol* 2005;45:2088–92.
41. Parry TJ, Brosius R, Thyagarajan R, et al. Drug-eluting stents: sirolimus and paclitaxel differentially affect cultured cells and injured arteries. *Eur J Pharmacol* 2005;524:19–29.
42. Poon M, Marx SO, Gallo R, et al. Rapamycin inhibits vascular smooth muscle cell migration. *J Clin Invest* 1996;98:2277–83.
43. Roque M, Reis ED, Cordon-Cardo C, et al. Effect of p27 deficiency and rapamycin on intimal hyperplasia: in vivo and in vitro studies using a p27 knockout mouse model. *Lab Invest* 2001;81:895–903.
44. Serruys PW, de Jaegere P, Kiemeneij F, et al. A comparison of balloon-expandable-stent implantation with balloon angioplasty in patients with coronary artery disease. Benestent Study Group. *N Engl J Med* 1994;331:489–95.
45. Sollott SJ, Cheng L, Pauly RR, et al. Taxol inhibits neointimal smooth muscle cell accumulation after angioplasty in the rat. *J Clin Invest* 1995;95:1869–76.
46. Stahl BE, Camici GG, Steffel J, et al. Paclitaxel enhances thrombin-induced endothelial tissue factor expression via c-Jun terminal NH2 kinase activation. *Circ Res* 2006;99: 149–55.
47. Steffel J, Hermann M, Greutert H, et al. Celecoxib decreases endothelial tissue factor expression through inhibition of c-Jun terminal NH2 kinase phosphorylation. *Circulation* 2005;111:1685–9.
48. Steffel J, Latini RA, Akhmedov A, et al. Rapamycin, but not FK-506, increases endothelial tissue factor expression: implications for drug-eluting stent design. *Circulation* 2005; 112:2002–11.
49. Steffel J, Luscher TF, Tanner FC. Tissue factor in cardiovascular diseases: molecular mechanisms and clinical implications. *Circulation* 2006;113:722–31.
50. Sun J, Marx SO, Chen HJ, et al. Role for p27(Kip1) in vascular smooth muscle cell migration. *Circulation* 2001;103:2967–72.
51. Suzuki T, Kopka G, Hayashi S, et al. Stent-based delivery of sirolimus reduces neointimal formation in a porcine coronary model. *Circulation* 2001;104:1188–93.
52. Togni M, Raber L, Cocchia R, et al. Local vascular dysfunction after coronary paclitaxel-eluting stent implantation. *Int J Cardiol* 2007;in press.
53. Urao N, Okigaki M, Yamada H, et al. Erythropoietin-mobilized endothelial progenitors enhance reendothelialization via Akt-endothelial nitric oxide synthase activation and prevent neointimal hyperplasia. *Circ Res* 2006;98:1405–13.
54. U.S. Food and Drug Administration CfDaRH. Cypher Sirolimus-eluting coronary stent on RAPTOR over-the-wire delivery system or RAPTORRAIL rapid exchange delivery system. Rockville: U.S. Food and Drug Administration CfDaRH, 2003 (available at <http://www.fda.gov/cdrh/pdf2/po20026.html>, accessed Dec 17th, 2004).
55. Wang TH, Wang HS, Ichijo H, et al. Microtubule-interfering agents activate c-Jun N-terminal kinase/stress-activated protein kinase through both Ras and apoptosis signal-regulating kinase pathways. *J Biol Chem* 1998;273:4928–36.
56. Windecker S, Simon R, Lins M, et al. Randomized comparison of a titanium-nitride-oxide-coated stent with a stainless steel stent for coronary revascularization: the TiNOX trial. *Circulation* 2005;111:2617–22.
57. Zhu S, Viswambharan H, Gajanayake T, et al. Sirolimus increases tissue factor expression but not activity in cultured human vascular smooth muscle cells. *BMC Cardiovasc Disord* 2005;5:22.

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